Response Surface Model for Predicting the Effects of Temperature pH, Sodium Chloride Content, Sodium Nitrite Concentration and Atmosphere on the Growth of *Listeria monocytogenes*

ABSTRACT

A factorial plus supplemental central composite experimental design was used to assess quantitatively the effects and interactions of temperature (5°-37°C), pH (4.5-7.5), NaCl (5-45 g/l), $NaNO_2$ (0-1000 μg/ml), and atmosphere (aerobic vs. anaerobic) on the growth kinetics of *Listeria monocytogenes* Scott A in Tryptose Phosphate Broth. A total of 709 growth curves were generated, with individual curves fitted using non-linear regression analysis in conjunction with the Gompertz function. The results were analyzed by response surface analysis to generate a cubic model to predict the growth of *L. monocytogenes* in response to any combination of the variables within the specified ranges. Evaluation of the model indicated that it can be used to provide reasonable "first estimates" of the impact of food formulation and storage conditions on the growth of *L. monocytogenes*, and can be automated readily to develop "user-friendly" software.

As consumers have increasingly demanded foods with minimal processing, there has been heightened interest in applying a multiple barrier approach to controlling the growth of foodborne microorganisms. However, actual application of this concept has been hampered by the lack of quantitative data that could be used to estimate the relative impact of the various factors that interact to influence microbial growth. This need, in combination with the ready availability of micro- and minicomputers, has stimulated interest in the development of predictive microbiology techniques that can be applied to food systems.

A number of approaches have been used to develop predictive equations that describe the effects of various cultural factors on the growth of selected foodborne pathogens (*7*). One that appears particularly promising is response surface regression analysis of data generated using the "Gompertz function" to quantitatively describe growth kinetics. This approach has been used successfully to empirically model the effects and interactions of temperature, pH, and sodium chloride content (a_w) on the growth of *Salmonella* (*1,9,14*).

The recent realization of the importance of foodborne transmission of *Listeria monocytogenes* in regard to the etiology of both epidemic and sporadic outbreaks of listeriosis has led to a need for data on how its growth characteristics are affected by various food formulation and storage factors. Our laboratory has been generating such data using model systems, and has demonstrated that storage temperature, initial pH, sodium chloride content, atmosphere, and sodium nitrite concentration interact to control the microorganisms's growth kinetics (*2*). The objective of the current study was to use these data to develop an empirical mathematical model(s) that effectively describes the effects and interactions of temperature (5-37°C), pH (4.5-7.5), sodium chloride (5-45 g/l), sodium nitrite (0-1000 μg/ml), and atmosphere (aerobic & anaerobic) on the growth kinetics of *Listeria monocytogenes*.

MATERIALS AND METHODS

Statistical design

The original data, developed using a factorial design (*2*), was supplemented with additional data derived using a central composite design (*3*). Complete data sets were generated for aerobic and anaerobic conditions. Subsequent experimentation to answer additional research questions was performed in a manner such that the data could be appended to the appropriate database.

Media

Tryptose Phosphate Broth (TPB) was used for all experimental cultures. Tryptose Phosphate Agar (TPA) (TPB + 2% Agar) was used for plate counts.

Microorganism

Listeria monocytogenes Scott A was used throughout the study. Stock cultures were maintained on TPA slants, and transferred monthly.

Culture techniques

The culture techniques employed were identical to those

described by Buchanan et al. (2). Briefly, media were prepared, supplemented with the appropriated level of NaCl, adjusted to the proper pH, transferred in 50 ml portions to 250 ml Erlenmeyer flasks (aerobic cultures) or 250 ml screw-cap trypsinizing flasks with a septum sealing the side-arm port (anaerobic cultures), and autoclaved. After cooling, the desired concentration of sodium nitrite was added as a filter-sterilized stock solution. Each flask was inoculated with 0.5 ml of a diluted 24 h culture of *L. monocytogenes*. The anaerobic cultures were then flushed with sterile N_2 for 10 min and sealed. All flasks were incubated in a shaker incubator at 150 rpm. Periodically, samples were removed by pipette (aerobic) or hypodermic needle and syringe (anaerobic) and surface plated in duplicate onto TPA using a Spiral Plater. All plates were incubated for 24 h at 37°C and then enumerated.

Curve fitting

Growth curves were generated for each experimental culture as described previously (2) using the Gompertz function (Table 1) in conjunction with ABACUS, an iterative non-linear regression program. This program was developed at the Eastern Regional Research Center by W. Damert, and is available upon request. The four Gompertz parameters (A, C, B, & M) were subsequently used to calculate exponential growth rates (EGR) ([log(cfu/ml)]/h), generation times (GT) (h), lag phase durations (LPD) (h), and maximum population densities (MPD) [log (cfu/ml)] (Table 1).

TABLE 1. *Equations for Gompertz function and derived growth kinetics values.*

Gomertz's Function:

$$L(t) = A + Ce^{-e^{(-B(t-M))}}$$

where:

L(t) = Log count of bacteria at time (in hours) t [Log(cfu/ml)].

A = Asymptotic log count of bacteria as time decreases indefinitely (i.e., initial level of bacteria [Log(cfu/ml)].

C = Asymptotic amount of growth that occurs as t increases indefinitely (i.e., number of log cycles of growth) [Log(cfu/ml)].

M = Time at which the absolute growth rate is maximal [h].

B = Relative growth rate at M. [(Log[cfu/ml])/h]

Derived Growth Kinetics Equations:

Exponential growth rate (EGR) = BC/e [Log(cfu/ml)/h]

Generation time (GT) = Log(2))e/BC [h]

Lag phase duration (LPD) = M - (1/B) [h]

Maximum population density (MPD) = A + C [Log(cfu/ml)]

Statistical analyses

Quadratic and cubic polynomial models in temperature, pH, sodium chloride content, and sodium nitrite concentration were calculated for "Gompertz" values and transformations of the Gompertz B and M terms were conducted using the SAS General Linear Model procedure.

RESULTS

A total of 392 aerobic and 317 anaerobic growth curves were generated over the course of approximately 18 months. Space considerations preclude presentation of the data; however, these databases are available upon request. The data expanded the findings of our earlier study (2) that temperature, initial pH, sodium chloride content, atmosphere, and sodium nitrite concentration interact to influence the growth kinetics of *L. monocytogenes*, particularly in regard to EGR/GT and LPD values.

Initial analysis indicated that the growth kinetics of *L. monocytogenes* were unaffected by the size of the initial inoculum. This was confirmed by supplemental experiments examining the effect of inocula between approximately 2×10^1 and 2×10^4 cfu/ml. For example, Fig. 1 depicts the Gompertz-fitted growth curves for aerobic cultures under conditions of 19°C, pH 7.5, 5 g/l NaCl, and 0 µg/ml $NaNO_2$. The Gompertz C, B, and M values varied linearly with A (inoculum size) in such a manner that the derived EGR, GT, LPD, and MPD values were constant. Analysis of the databases also indicated that, except for extremes of cultural conditions, if the microorganism grew it attained population densities of between 2×10^8-2×10^{10} cfu/ml (Fig. 2). These results indicated that (*a*) the average values for MPD could be used as a constant (aerobic = 9.57, anaerobic = 9.34), (*b*) the Gompertz C value was largely unaffected by the independent variables, and (*c*) the Gompertz A and C values could be ignored during subsequent model development. Similar conclusions were reached by Gibson et al. (9) during the development of their Gompertz-based model for *Salmonella*.

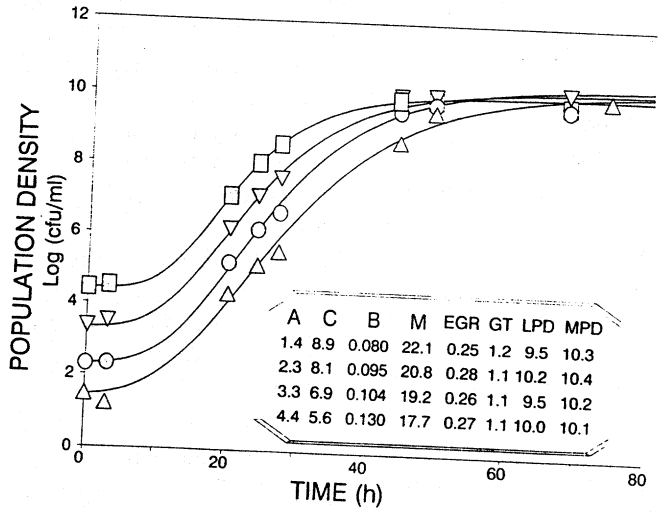

Figure 1. *Effect of inoculum size on the growth kinetics of* L. monocytogenes *Scott A incubated aerobically in tryptose phosphate broth (pH 7.5, 5 g/l NaCl, 0 µg/ml $NaNO_2$) at 19°C. Growth curves were generated using "best fit" Gompertz curve. See Table 1 for abbreviations.*

Since consideration of atmospheric composition was restricted to aerobic (air) versus anaerobic (nitrogen-flushed), it was concluded that development of effective models

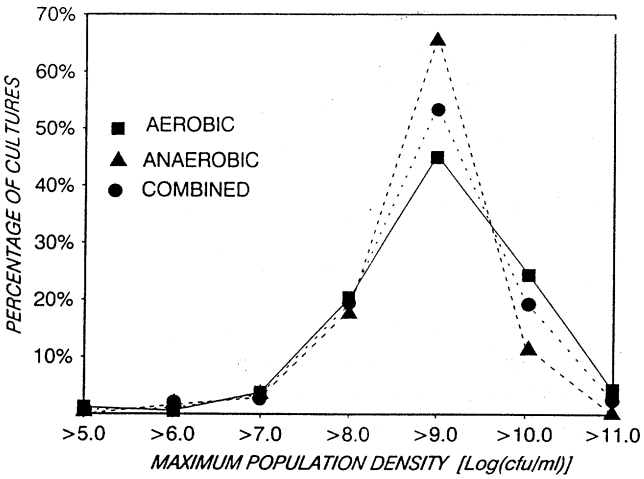

Figure 2. *Frequency distributions of aerobic, anaerobic, and combined aerobic + anaerobic data for maximum population densities attained by cultures of* L. monocytogenes *Scott A in conjunction with various combinations of temperature, pH, NaCl content, and* $NaNO_2$ *concentrations.*

would be simpler by analyzing the aerobic and anaerobic databases independently. Accordingly, separate sets of response surface equations were developed for each atmospheric condition.

Initial evaluation of the databases used a quadratic polynomial response surface analysis of the Gompertz B and M values without any transformations as functions of temperature, pH, sodium chloride content, and sodium nitrite concentration. In this instance the no-growth data had to be eliminated from consideration, since the M values were equal to infinity. The derived equations gave reasonable fits with the experimental data when the cultural conditions were close to optimal, but gave increasingly poorer (and, at time, nonsensical) values under decreasingly optimal variable combinations. Inclusion of the no-growth data was attempted by using a 1/M transformation; 1/M approaches zero as M approaches infinity. Using all of the data yielded a somewhat better fit; however, there were still relatively poor fits of predicted versus observed data under less than optimal growth conditions.

After several additional transformations were evaluated, it was concluded that the Ln transformation described by Gibson et al. (*9*) was the most effective. This transformation was used to analyze the data using a quadratic polynomial model both with (not shown) and without (Table 2) the inclusion of the no-growth data. The no-growth data were included using a Ln [(1/M) + 0.001] and Ln (B + 0.001) transformation; the constant 0.001 being added to avoid B or 1/M values of 0.000 that could not be analyzed using a Ln function. Since a number of potential three-way interactions were apparent, it was decided to also evaluate the data using a cubic model with all two- and three-way cross products. Again, the data were analyzed with (not shown) and without (Table 3) the inclusion of the no-growth data.

The four sets of equations were evaluated by both

TABLE 2. *Quadratic models for the effects and interactions of temperature (T) (°C), initial pH (P), sodium chloride content (S) (g/l), and sodium nitrite concentration (N) (μg/ml) on the aerobic and anaerobic growth of* Listeria monocytogenes *Scott A, using Ln(M) and Ln(B) transformations in conjunction with variables combinations producing no-growth responses being treated as missing values.*

Aerobic

$$Ln(M) = 20.454 - 0.2574T - 4.3526P + 0.0173S + 0.0198N + 0.0028T^2 + 0.0326P^2 + 0.000205S^2 + 0.00000012N^2 + 0.00477TP + 0.000279TS + 0.00000719TN - 0.00335PS - 0.00267PN + 0.00000094SN$$

Degrees of freedom =302

$R^2 = 0.962$

$$Ln(B) = -18.4243 + 0.3155T + 3.6855P + 0.0366N - 0.00316T^2 - 0.2622P^2 - 0.000443S^2 - 0.00000007N^2 - 0.0144TP - 0.000177TS - 0.00000275TN - 0.00275PS + 0.00266PN - 0.00000533SN$$

Degrees of freedom = 302

$R^2 = 0.927$

Anaerobic

$$Ln(M) = 18.9022 - 0.1734T - 3.9898P + 0.0385S + 0.025N + 0.00312T^2 + 0.3204P^2 - 0.000121S^2 - 0.00000048N^2 - 0.00801TP + 0.00011PS + 0.0000121TN - 0.00372PS - 0.00328PN - 0.00000225SN$$

Degrees of freedom = 223

$R^2 = 0.952$

$$Ln(B) = -8.7529 + 0.098T + 1.427P - 0.039S - 0.0188N - 0.00277T^2 - 0.1338P^2 + 0.0003S^2 + 0.00000334N^2 + 0.0167TP - 0.000257TS - 0.00000712TN + 0.00417PS + 0.00248PN - 0.000000197SN$$

Degrees of freedom = 223

$R^2 = 0.906$

examination of residuals and direct comparison of predicted values against a standardized set of experimental values. In general, the models without the inclusion of the no-growth data yielded a better fit with the experimental data, particularly for non-optimal growth conditions. Apparently, the no-growth data tended to bias the equations which is not unreasonable considering that these data were of course clustered in the non-optimal growth regions of the array of variables. Of the two models derived without the inclusion of no-growth data, the cubic model gave a significantly better fit between predicted and observed values than the quadratic model. This was not unexpected in that a number of highly significant interactions between variables was observed. Examples of values predicted by the cubic model and observed values for several variable combinations are depicted in Table 6.

The quadratic and cubic equations were submitted to stepwise regression using the backward elimination method which removed non-significant variables and reanalyzed the data. Evaluation of the "streamlined" quadratic (not shown) and cubic (Table 4) equation sets again indicated that a substantially better fit could be achieved using the cubic models. The F-values observed with the cubic model

TABLE 3. *Cubic models for the effects and interactions of temperature (T) (°C), initial pH (P), sodium chloride content (S) (g/l), and sodium nitrite concentration (N) (μg/ml) on the aerobic and anaerobic growth of* Listeria monocytogenes *Scott A, using Ln(M) and Ln(B) transformations in conjunction with variables combinations producing no-growth responses being treated as missing values.*

Aerobic

$$Ln(M) = 37.657 + 0.0135T - 13.7331P + 0.4013S + 0.0713N + 0.00375T^2 + 1.9759P^2 - 0.000667S^2 - 0.000007051N^2 - 0.083TP + 0.000842TS - 0.0002141TN - 0.1155PS - 0.0167PN - 0.000125SN + 0.0000292T^3 - 0.0935P^3 + 0.00000328S^3 + 0.000286TPS + 0.0000315TPN + 0.00000014TSN + 0.0000175PSN - 0.000384T^2P - 0.00000855T^2S - 0.00000043T^2N + 0.00731TP^2 - 0.0000441TS^2 + 0.00672P^2S + 0.000968P^2N + 0.000294PS^2 + 0.00000062PN^2 - 0.00000016S^2N$$

Degrees of freedom = 308

$R^2 = 0.967$

$$Ln(B) = -47.709 + 0.1631T + 18.6861P - 0.3609S + 0.01N - 0.00161T^2 - 2.7074P^2 + 0.00623S^2 - 0.0000863N^2 + 0.0242TPS - 0.000906TS + 0.000594TN + 0.0671PS - 0.00715PN + 0.000337SN - 0.0000648T^3 + 0.1276P^3 - 0.000029S^3 - 0.000551TPS - 0.0000733TPN - 0.00000033TSN - 0.0000431PSN + 0.000189T^2P + 0.0000549T^2S - 0.00000047T^2N - 0.00222TP^2 + 0.0000459TS^2 - 0.00000002TN^2 - 0.000781P^2S + 0.000777P^2N - 0.000872PS^2 + 0.0000112PN^2 - 0.00000038S^2N$$

Degrees of freedom = 308

$R^2 = 0.942$

Anaerobic

$$Ln(M) = 89.9195 - 0.5378T - 38.8065P + 1.735S + 0.2175N + 0.00284T^2 + 5.9583P^2 + 0.00962S^2 - 0.000186N^2 + 0.1063TP - 0.00159TS + 0.000397TN - 0.567PS - 0.0574PN + 0.0000813SN - 0.0000321T^3 - 0.3024P^3 - 0.000107S^3 - 0.0000148TPS - 0.0000468TPN - 0.00000118TSN - 0.0000143PSN + 0.000397T^2P - 0.0000126T^2S - 0.00000184T^2N - 0.00964TP^2 + 0.0000487TS^2 + 0.0000001TN^2 + 0.0436P^2S + 0.0038P^2N - 0.000461PS^2 + 0.0000247PN^2 + 0.00000123S^2N - 0.00000001SN^2$$

Degrees of freedom = 211

$R^2 = 0.974$

$$Ln(B) = -78.2567 + 0.7928T + 34.3598P - 0.913S - 0.4437N + 0.00218T^2 - 5.3119P^2 - 0.00394S^2 + 0.000233N^2 - 0.2134TP - 0.00174TS - 0.00094TN + 0.3002PS + 0.1272PNS - 0.00015SN + 0.0000274T^3 + 0.2693P^3 + 0.0000493S^3 + 0.000442TPS + 0.0000985TPN - 0.00000047TSN + 0.0000304PSN - 0.00104T^2P + 0.00000175T^2S + 0.00000584T^2N + 0.0194TP^2 - 0.0000318TS^2 - 0.00000011TN^2 + 0.0238P^2S - 0.00911P^2N + 0.000215PS^2 - 0.0000298PN^2 - 0.00000068S^2N - 0.00000003SN^2$$

Degrees of freedom = 211

$R^2 = 0.944$

obtained by stepwise elimination are presented in Table 5. Examples of predicted values for the full and reduced cubic models compared to observed experimental data are depicted in Table 6. The full cubic model gave an overall better fit than the reduced revision. Comparison of predicted versus published grow kinetics values for various foods and media systems are presented in Table 7.

DISCUSSION

Though the databases were too large for direct inclusion, there were several growth characteristics of the microorganism worth further mention. First, there were a number of instances when growth was observed in cul-

TABLE 4. *Cubic models for the effects and interactions of temperature (T) (°C), initial pH (P), sodium chloride content (S) (g/l), and sodium nitrite concentration (N) (μg/ml) on the aerobic and anaerobic growth of* Listeria monocytogenes *Scott A, after equations in Table 3 were submitted to stepwise regression using the backward elimination method.*

Aerobic

$$Ln(M) = 31.1837 - 10.5756P + 0.5384S + 0.0193N + 0.00547T^2 - 1.4851P^2 - 0.0877TP - 0.1598PS - 0.00256PN - 0.0689P3 + 0.000333TPS - 0.000404T^2P + 0.00781TP^2 - 0.0000406TS^2 + 0.0104P^2S + 0.000201PS^2$$

$R^2 = 0.965$

n = 308

$$Ln(B) = -48.5373 + 0.2158T + 18.702P - 0.2994S - 2.6591P^2 + 0.00426S^2 - 0.0000788N^2 + 0.000379TN + 0.0571PS - 0.00355PN + 0.000325SN - 0.000069T^3 - 0.1226P^3 - 0.000572TPS - 0.0000505TPN - 0.0000445PSN + 0.0000502T^2S + 0.0000337TS^2 + 0.00048P^2N - 0.000867PS^2 + 0.0000105PN^2$$

$R^2 = 0.941$

n = 308

Anaerobic

$$Ln(M) = 75.5452 - 0.2025T - 32.4453P + 1.1523S + 0.263N + 0.00416T^2 + 5.0087P^2 + 0.00485S^2 - 0.000165N^2 - 0.00117TS - 0.3649PS - 0.00703PN + 0.000173SN - 0.0000405T^3 - 0.2546P^3 - 0.0000763S^3 - 0.00000127TSN - 0.000019PSN + 0.000262T^2P - 0.0000121T^2S - 0.0015TP^2 + 0.000038TS^2 + 0.00000005TN^2 + 0.0269P^2S + 0.00471P^2N + 0.0000218PN^2$$

$R^2 = 0.974$

n = 211

$$Ln(B) = -3.9926 + 0.1248T - 0.4613N - 0.0321P^2 + 0.000242N^2 - 0.00164TS - 0.001TN + 0.1322PN + 0.0000498T^3 + 0.0000143S^3 + 0.000425TPS + 0.000425TPS + 0.000108TPN - 0.000913T^2P + 0.00000536T^2N + 0.00315TP^2 - 0.0000315TS^2 - 0.0000001TN^2 - 0.000545P^2S - 0.00942P^2N - 0.0000318PN^2$$

$R^2 = 0.942$

n = 211

TABLE 5. *F-values for significant variables for cubic model after stepwise regression using the backward elimination method.*

Variable	Aerobic		Anaerobic	
	Ln(M)	Ln(B)	Ln(M)	Ln(B)
INTERCEPT	17.9(0.0001)[a]	26.2(0.001)	10.9(0.0011)	171.6(0.001)
T	----[b]	1428.6(0.0001)	40.9(0.0001)	73.3(0.0001)
P	7.5(0.0067)	14.5(0.0002)	8.9(0.0032)	----
S	15.2(0.0001)	18.7(0.0001)	3.4(0.0655)	----
N	140.3(0.0001)	----	5.9(0.0161)	35.3(0.0001)
T^2	28.7(0.0001)	----	37.7(0.0061)	----
P^2	5.0(0.0255)	10.4(0.0014)	8.5(0.0039)	19.2(0.0001)
S^2	----	10.6(0.0013)	3.4(0.0659)	----
N^2	----	4.4(0.0351)	44.1(0.0001)	37.6(0.0001)
T*P	156.0(0.0001)	----	----	----
T*S	----	----	3.3(0.0719)	8.9(0.0031)
T*N	----	3.8(0.0535)	----	8.0(0.0050)
P*S	14.7(0.0001)	25.3(0.0001)	3.4(0.0660)	----
P*N	138.4(0.0001)	7.6(0.0062)	4.7(0.0317)	32.6(0.0001)
S*N	2.9(0.0884)	7.3(0.0072)	4.1(0.0437)	----
T^3	----	336.2(0.0001)	5.9(0.0160)	7.6(0.0063)
P^3	3.5(0.0629)	7.3(0.0072)	8.0(0.0050)	----
S^3	----	----	4.7(0.0317)	9.9(0.0019)
N^3	----	----	----	----
T*P*S	31.9(0.0001)	60.3(0.0001)	----	15.2(0.0001)
T*P*N	----	3.8(0.0534)	----	5.1(0.0257)
T*S*N	----	----	17.3(0.0001)	----
P*S*N	----	7.6(0.0061)	2.8(0.0983)	----
T^2*P	7.3(0.0071)	----	2.8(0.0969)	30.2(0.0001)
T^2*S	----	38.2(0.0001)	4.0(0.0463)	----
T^2*N	----	----	----	12.8(0.0004)
T*P^2	60.4(0.0001)	----	8.2(0.0046)	31.5(0.0001)
T*S^2	24.9(0.0001)	9.5(0.0022)	9.7(0.0021)	4.4(0.0378)
T*N^2	----	----	21.0(0.0001)	5.0(0.0268)
P^2*S	11.1(0.0009)	----	3.4(0.0671)	7.1(0.0083)
P^2*N	----	7.6(0.0061)	3.8(0.0519)	30.3(0.0001)
P*S^2	30.2(0.0001)	16.5(0.0001)	----	----
P*N^2	----	4.5(0.0354)	43.2(0.0001)	36.6(0.0001)
S^2*N	----	----	----	----
S*N^2	----	----	----	----

[a]F-Value (probability of a larger value of F).
[b]Variable eliminated as non-significant (P>0.10).

tures having an initial pH = 4.5. While *L. monocytogenes* has been generally considered to be relatively sensitive to acid conditions, George et al. (*8*) and Parish and Higgins (*12*) recently observed growth at pH 4.4 and 4.5, respectively. Those studies along with the current data suggest that, at least in model systems, the microorganism can tolerate reasonably acid conditions. Further, our data to date is consistent with the observations of George et al. (*8*) that the ability of *L. monocytogenes* to grow at low pH levels is highly dependent on incubation temperature. Additional studies are underway to confirm these observations. Secondly, the additional data generated using nitrogen flushed cultures reinforce further the conclusion of Buchanan et al. (*2*) that *L. monocytogenes* is highly suited to anaerobic conditions. In the absence of nitrite, the microorganism's growth kinetics were similar for aerobic and aerobic conditions. Finally, the supplemental data further confirmed that the primary inhibitory action against *L. monocytogenes* involves a bacteriostasis, resulting in an extension of the lag phase and a depression of the growth rate. Depression of maximum population densities was largely restricted to the more extreme culture conditions. This indicates that Shahamat et al. (*17*), who measured the antimicrobial activity of nitrite by measuring maximum population density depression, underestimated the effectiveness of nitrite against *L. monocytogenes*. Recent studies by Junttila et al. (*10*) indicated that nitrite accelerated the inactivation of *L. monocytogenes* is fermented meats.

While a number of conclusions reached in conjunction with the development of effective response surface models for *L. monocytogenes* were similar to those for *Salmonella*, there were substantial differences. Similar to Gibson et al. (*9*), stabilizing the variability through the use of a Ln transformation was found to be critical. Our inability to enhance the accuracy of the models through the use of reciprocal transformations so that the no-growth data could be included was disappointing. We are continuing to investigate alternate transformations that could be used to

TABLE 6. *Comparison of selected examples of experimentally observed vs predicted values for the cubic models, with and without stepwise regression using the backward elimination method (Table 3 and 4), of the growth of* Listeria monocytogenes *Scott A.*

				Experimental Values				Full Cubic Models (Table 5)			Reduced Cubic Models (Table 7)		
(°C)	pH	(%)	(μg/ml)	Atm	EGR (log(cfu/ml)/h)	GT (h)	LPD (cfu/ml)	EGR (log(cfu/ml)/h)	GT (h)	LPD (cfu/ml)	EGR (log(cfu/ml)/h)	GT (h)	LPD (cfu/ml)
5	5.50	0.5	0	A	0.022	13.8	106.0	0.021	14.2	80.4	0.020	14.8	88.2
5	6.00	4.5	100	A	0.008	37.9	213.0	0.012	24.2	214.1	0.013	23.4	263.3
5	7.00	0.5	0	N	0.022	13.5	55.0	0.020	14.9	36.4	0.025	12.1	59.3
5	6.00	2.5	100	N	0.016	19.1	305.6	0.019	16.0	286.6	0.018	17.1	286.8
10	6.75	1.5	50	N	0.065	4.6	11.7	0.062	4.8	14.5	0.061	4.9	14.4
19	5.25	0.5	0	A	0.352	0.9	8.9	0.187	1.6	13.8	0.178	1.7	14.4
19	7.50	4.5	200	N	0.288	1.1	16.4	0.201	1.5	15.7	0.175	1.7	10.7
28	6.00	0.5	100	N	0.170	1.8	8.1	0.174	1.7	7.4	0.169	1.8	7.1
28	7.50	4.5	0	A	0.339	0.9	5.1	0.263	1.2	5.3	0.153	2.0	-0.8
37	6.00	0.5	200	N	0.306	1.4	10.4	0.320	0.9	11.5	0.272	1.1	10.1
37	7.50	4.5	200	A	0.396	0.8	4.6	0.321	0.9	4.8	0.116	2.6	-8.2

TABLE 7. *Comparison of selected reported growth kinetics for* Listeria monocytogenes *versus those predicted by the cubic models*[a].

Food	(°C)	pH	(%)	Generation Time (h)		Lag Phase (h)		Reference
				Reported	Predicted	Reported	Predicted	
1. Clarified cabbage juice	30	6.1	0.5	1.6-1.8	0.4	10	3	Conner et al. (*4*)
2. Clarified cabbage juice	30	6.1	2.0	2.2-2.3	0.5	---[d]	3	Conner et al. (*4*)
3. Whole milk	10	(6.7)[b]	(0.5)	10	4.4	24	25	Marshall & Schmidt (*11*)
4. Chocolate milk	13	(6.7)	(2.5)[c]	3.9-4.7	2.1	----	12	Rosenow & Marth (*15*)
5. 2% Milk	13	(6.7)	(0.5)	4.4-4.5	2.6	12	16	Rosenow & Marth (*15*)
6. Uncultured whey	6	6.2	(0.5)	14.8-21.1	8.9	72	50	Ryser & Marth (*16*)
7. Cultured whey	6	6.8	(0.5)	16.3-17.4	9.4	72	48	Ryser & Marth (*16*)
8. Ice cream mix	9.5	6.4	(4.5)[c]	8.7-13.3	7.5	22-95	33	Smith & Holsinger (unpublished data)
9. Whole milk	9.5	6.7	(0.5)	5.2-9.0	4.8	7-12	27	Smith & Holsinger (unpublished data)
10. Tryptose broth	4	5.6	0.5	27.1	16.3	144	91	El-Shenawy & Marth (*5*)
11. Typtose broth	21	5.0	0.5	8.0	1.8	12	16	El-Shenawy & Marth (*5*)
12. Tryptose broth	35	5.0	0.5	1.1	1.1	6	9	El-Shenawy & Marth (*5*)
13. Tryptose broth	35	5.6	0.5	0.8	0.5	3	3	El-Shenawy & Marth (*5*)
14. Tryptic soy broth	4	7.0	0.5	33.5	14.7	---	68	Petran & Zottola (*13*)
15. Tryptic soy broth	13	7.0	0.5	4.8	2.8	---	17	Petran & Zottola (*13*)
16. Tryptic soy broth	35	7.0	0.5	0.7	0.5	---	1	Petran & Zottola (*13*)
17. Tryptic soy broth	30	4.7	0.5	6.2	1.7	---	19	Petran & Zottola (*13*)
18. Tryptose phosphate broth	4	5.6	0.5	26.4	16.2	144	91	El-Shenawy & Marth (*6*)
19. Tryptose phosphate broth	4	5.0	0.5	NG[e]	29.9	NG	144	El-Shenawy & Marth (*6*)
20. Tryptose phosphate broth	13	5.0	0.5	8.0	5.4	12	38	El-Shenawy & Marth (*6*)
21. Tryptose phosphate broth	21	5.6	0.5	1.8	0.9	6	8	El-Shenawy & Marth (*6*)
22. Tryptose phosphate broth	35	5.6	0.5	0.8	0.5	3	3	El-Shenawy & Marth (*6*)

[a]All food samples were assumed to be aerobic.
[b]Parentheses indicate that value not given and had to be assumed.
[c]An elevated value was assumed to estimate effect of ingredients other than NaCl that would affect the water activity of the food system. The ice cream mix samples had measured a (10°C) values of 0.930-0.956.
[d]Value not reported.
[e]No growth.

take advantage of this class of data, but based on our experiences, it seems reasonable to recommend that the selection of experimental combinations for generating Gompertz-based growth data avoid no-growth responses. Since the variability of the data tended to increase the further conditions deviated from optimal, a central composite design which assumes a constant variation may not be the most appropriate experimental design. Though the Ln transformation helped stabilize the variation, alternate designs that increase the number of replicates of non-optimal variable combinations may prove to be more effective. This is, in part, the reason why a combined factorial/central composite design was employed in the current study. Further work into optimizing experimental designs is needed to ensure maximal usefulness of the data collected, particularly considering the labor intensive experimentation required to generate the values.

Using a random data elimination procedure, Bratchell et al. (*1*) concluded that modeling the B-values for *Salmonella* was substantially more sensitive to variation than the M values. While the *L. monocytogenes* data have not been subjected to such a detailed analysis, our experience has been generally opposite in that modeling the M values, particularly when associated with non-optimal conditions, was more difficult. The reason for this difference is not clear but could reflect differences in the software used to fit the Gompertz curves to the experimental data, the expansion of data ranges into more non-optimal conditions (e.g., psychrotrophic temperature, inhibitory activity of nitrite, etc.), or the inclusion of additional variables (i.e., four instead of three dimensions). The difference in the two studies is reflected in the need in the current study to take the response surface models to a higher order (cubic vs. quadratic) to achieve an effective fit with the experimental data. While the approach with both microorganisms involved an empirical fit of the data, Roberts (*14*) suggested that the quadratic model of the *Salmonella* data could be viewed as a Taylor series approximation of true underlying functions. The current study suggests that, at least for *L. monocytogenes* an underlying quadratic relationship could not be assumed. Instead, it appears more important to select a model that takes into account the degree of interaction among the variables. In the current study, the inclusion of nitrite as a factor and its highly significant three-way linear and two-way quadratic interactions (Table 5) with other variables predisposed the data for higher level modeling.

The effectiveness of a model is ultimately dependent on its ability to predict "real world" data. Comparison of predicted values against published growth kinetics data for *L. monocytogenes* (Table 7) indicated that the full cubic models provided reasonable predictions of the organism's behavior in food systems. The differences between the predicted and reported values tended to be greater for LPD than EGR/GT values. This may be partially due to the greater inherent variability of the physiological processes occurring during the lag phase. Further, several of the cited studies appear to have used a highly empirical method for estimating reported LPD values. The models were generally conservative in that they tended to indicate that the organism was more actively capable of growing under adverse conditions than was actually the case. This is not surprising considering that there were likely additional factors not reflected in the model that influenced the growth of the microorganism in the food systems. One of the underlying reasons for electing to develop models based on data generated using a microbiological medium system was that the models would tend to provide a "worst case scenario" in regard to growth potential of *L. monocytogenes*. Overall, the models appear to provide reasonable "first round estimates" that should be very useful in terms of allowing food microbiologists and individuals involved in new product development to assess quickly the impact of altering any combination of the variables. The same approach could be used to generate models for data developed for specific food systems.

In addition to the scientific attributes of various models that have been researched, a critical factor affecting the ultimate utility of a model is the ease with which it can be adapted for use by non-research personnel. Too often good work in predictive microbiology has gone unused due to lack of appropriate application methods. Models based on the use of the Gompertz function lend themselves readily to the development of "user-friendly" applications software, and is a reason why both the current study and Gibson et al. (*9*) elected to model the Gompertz values and not the derived growth kinetics values for EGR, GT, LPD, and MPD. Using a commercially available spreadsheet program, we found it relatively easy to develop a highly user-friendly program that incorporates both the current model for *L. monocytogenes* and that of Gibson et al. (*9*) for *Salmonella*. The development of this software will be described in a separate publication and will be made available to interested parties.

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